Key Characteristics of Carcinogens and an Approach to using Mechanistic Data in their Classification

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Introduction

Recently, the International Agency for Research on Cancer (IARC) completed a review of all its known Group 1 human carcinogens and updated information on tumor sites and mechanisms of carcinogenesis (IARC Monograph Volumes100A-F). About half of the agents classified in Group 1 were last reviewed more than 20 years ago, before mechanistic studies became prominent in evaluations of carcinogenicity. In addition, more recent epidemiological studies and animal cancer bioassays have demonstrated that many cancer hazards reported in earlier studies were later observed in other organs or through different exposure scenarios.

In compiling this information for Volumes 100A-F, two things became apparent. First, no systematic method for collating and analyzing mechanistic information was readily available, and second, that the agents documented and listed as known human carcinogens showed a number of key characteristics that distinguish them as carcinogenic agents. Many appear to act via multiple mechanisms causing various biological changes in the multistage process of carcinogenesis. Others appear to act by a single predominant mechanism.

In 2012, participants at two workshops at IARC in Lyon, France extensively debated the mechanisms by which carcinogens produce cancer. The Group concluded that carcinogens frequently exhibit one or more of 10 key characteristics, as described in Table 1. Herein we define these 10 key characteristics and discuss their importance in the carcinogenic process. These characteristics are not mechanisms, modes of action or adverse outcome pathways, but are properties that human carcinogens commonly show. They are also not hallmarks of cancer, which are the properties of cancer cells and neoplasms and not of the agents that cause cancer.

Furthermore, we also describe how the 10 key characteristics can provide a basis for systematically collating and analyzing mechanistic information as part of the carcinogen evaluation process. The U.S. EPA, as well as IARC, have both recognized a need for such an approach. Indeed, in evaluating EPA's IRIS (Integrated Risk Information System) Program, the U.S. National Research Council emphasized the need for consistent, transparent, systematic approaches for the identification, evaluation, and integration of data for IRIS assessments, in keeping with recent efforts by the IRIS Program.

However, mechanistic study databases present a challenge to systematic reviews in that the studies are typically both numerous and diverse, involving a multitude of targets and toxicity pathways. For example, the mechanistic study database identified by preliminary literature searches for benzene contains over 1,800 studies, many with multiple mechanistic endpoints. An approach for comprehensively identifying the relevant literature was initiated, based on systematic searches for outcomes pertinent to the 10 key characteristics of human carcinogens. The search terms, sources and results were documented, and the resulting literature categorized according to the key characteristics associated with the reported outcomes. This categorization facilitated objective consideration of the relevant mechanistic information, thereby advancing analyses of hypothesized mechanisms and toxicity pathways. Since mechanistic data may provide evidence of carcinogenicity, and can play a role in up- or downgrading an evaluation based on cancer findings in animals, it is hoped that this systematic approach and set of established characteristics will assist future IARC Working Groups and other agencies in evaluating additional potential human carcinogens.

Description of the Key Characteristics

Characteristic 1: Electrophilic or can be Metabolically Activated

Electrophiles are electron-seeking molecules that commonly form addition products, commonly referred to as adducts, with cellular macromolecules including DNA, RNA and proteins. Some chemical carcinogens are direct-acting electrophiles, whereas others require biotransformation by enzymes in a process termed metabolic activation (Miller 1970). Examples of direct-acting electrophilic carcinogens include sulfur mustards and ethylene oxide (Rusyn, Asakura et al. 2005, Grosse, Baan et al. 2007, Humans 2008, Batal, Boudry et al. 2014). The classic examples of chemical agents that require metabolic activation to become carcinogenic include polycyclic aromatic hydrocarbons and benzene, which by themselves are relatively chemically inert (Slaga, Fischer et al. 1980, Smith 1996). This lack of reactivity puzzled chemists working on experimental carcinogenesis for many years, until the Millers discovered metabolic activation by the mixed function oxidase system (Conney, Miller et al. 1957). It is now known that a number of human enzymes can bio-transform relatively inert chemical compounds to potent toxic and carcinogenic metabolites or reactive intermediates, including cytochrome P450s, flavin mono-oxygenase, prostaglandin synthetase and various peroxidases (O'Brien 2000, Hecht 2012). The ability to form adducts on nucleic acids and proteins is a common property of these inherently electrophilic and/or metabolically activated human carcinogens (Ehrenberg 1984).

Characteristic 2: Genotoxic

The term "genotoxic" refers to an agent that induces DNA damage. Most of the IARC Group-1 human carcinogens are considered to be genotoxic and many are mutagenic (Waters, Jackson et al. 2010), though this may not be their primary mechanism of carcinogenesis. DNA damage from genotoxicity may be in the form of DNA adducts or single- or double-strand breaks. Evidence of genotoxicity comprises DNA damage, gene mutation per se, or chromosomal damage. DNA damage includes DNA adducts, strand breaks, or cross links, as well as the fragmentation of bases or the intercalation of a molecule between a pair of bases. The DNA damage is by itself not a mutation and generally does not alter the linear sequence of nucleotides (or bases) in the DNA. Gene mutation is a change in the DNA sequence and usually arises as the cell attempts to

repair the DNA damage. Structural or numerical alterations in chromosomes can also arise as a consequence of DNA damage and subsequent repair and can be lethal to the cell as well as mutagenic. Chromosomal damage can manifest itself in many ways, including as micronuclei, aneuploidy, loss of heterozygosity and intrachromosomal recombination.

Characteristic 3: Alter DNA Repair or Cause Genomic Instability

Normal cells avoid deleterious mutations by replicating their genomes with high accuracy. However, DNA replication fidelity can vary widely depending on the DNA polymerase involved, introducing the possibility of error. Indeed, most spontaneous mutations are caused by polymerase error (Preston, Albertson et al. 2010). The nature of the error, the flanking sequence, the presence of DNA damage and the ability to correct errors all impact on the outcome of this process (Arana and Kunkel 2010). As a consequence, defects in processes that determine DNA-replication fidelity can confer strong mutator phenotypes that result in genomic instability. Thus, carcinogens may act not only by producing DNA damage directly, but also by altering the processes that control normal DNA replication.

Similarly, the major DNA-repair pathways such as base-excision repair (BER), nucleotide-excision repair (NER) and double-strand break (DSB) repair involved in the removal of DNA adducts and other lesions may also be altered by environmental exposures or inherited genetic polymorphisms. Further, while especially excision-repair pathways are predominantly error-free and thus protective, DSB repair is largely error-prone and may contribute to genomic instability.

Genomic instability is a well-recognized feature of many cancers (Bielas, Loeb et al. 2006). Studies of cellular radiation exposure have shown that instability is a relatively late-occurring event that appears several cell generations after irradiation and results in a reduced ability to replicate the genotype faithfully (Kadhim, Salomaa et al. 2012). The events indicating genomic instability include chromosome aberrations, gene mutations, microsatellite instability, and apoptosis. It is possible that the instability phenotype may play a major role in radiation-induced and other forms of cancer by providing the cell and its progeny with a persistent elevation in the rate of numerous and varied genetic and epigenetic changes that may occur in multistage carcinogenesis (Aypar, Morgan et al. 2011).

Characteristic 4: Induce Epigenetic Alterations

The term “epigenetic” refers to all stable changes in gene expression and chromatin organization that are independent of the DNA sequence itself and that can be mitotically inherited over cell divisions (Herceg, Lambert et al. 2013). Epigenetic phenomena, including genomic imprinting, X-chromosome inactivation and global reconfiguration of the DNA methylome, changes in chromatin compaction states, and histone modification patterns, occur during development and contribute to the lineage-specific epigenome that is maintained over the lifetime of an organism. Many of these same phenomena have been shown to be altered during carcinogenesis (Herceg, Lambert et al. 2013). A wide range of known and suspected carcinogens (including chemical, physical and biological agents) have been shown to deregulate the epigenome, and it has been suggested that their mode of action may involve disruption of epigenetic mechanisms. However, evidence for a truly causal role of epigenetic changes in cancer produced by

Group 1 agents was considered to be limited in Volume 100, and for many agents, their impact on the epigenome was considered to be a secondary mechanism of carcinogenesis. Herceg (Herceg, Lambert et al. 2013) and others have described a wealth of studies demonstrating the impact of many of the Volume 100 carcinogens on epigenetic mechanisms. They note, however, that most carcinogens (even those evaluated for Volume 100 in 2008 and 2009) were evaluated by IARC Working Groups before new data on their epigenetic effects became available. This rapidly evolving area will generate new mechanistic data on carcinogens in the next few years.

Characteristic 5: Oxidative stressor

Many human and animal carcinogens are capable of influencing redox processes and balance within target cells. If an imbalance in reactive oxygen and/or nitrogen species formation and their detoxification is produced, this is commonly referred to as oxidative stress. Reactive oxygen species arising from tissue inflammation, xenobiotic metabolism, interruption of mitochondrial oxidative phosphorylation (Figueira, Barros et al. 2013), or reduced turnover of oxidized cellular components may contribute to genomic instability. These and other free radical species play key roles in many of the processes identified as being necessary for the conversion of normal cells to cancer cells. Oxidative damage is considered a major factor in the generation of mutations in DNA and over 100 different oxidative DNA adducts have been identified (Klaunig, Wang et al. 2011). At least 24 base modifications are produced by reactive oxygen species, as well as DNA-protein crosslinks and other lesions (Berquist and Wilson 2012), all potentially leading to genomic instability. Oxidative damage to DNA can lead to point mutations, deletions, insertions, or chromosomal translocations, which may cause oncogene activation and tumor suppressor gene inactivation, and potentially initiate or advance carcinogenesis (Klaunig, Wang et al. 2011, Berquist and Wilson 2012). Thus, agents that generate and promote oxygen radical-induced cellular injury may be carcinogenic.

Characteristic 6: Induce Chronic Inflammation

Chronic inflammation from persistent infections, such as that produced by *H. pylori*, as well as that produced sterilely by silica or asbestos fibers, has been associated with several forms of cancer (Grivennikov, Greten et al. 2010). Indeed, inflammation has been hypothesized to contribute to multiple aspects of cancer development and progression (Trinchieri 2012). Inflammation acts by both intrinsic and extrinsic pathways. Persistent infection and chronic inflammation disrupt local tissue homeostasis and alter cell signaling, leading to the recruitment and activation of inflammatory cells. These constitute extrinsic pathways linking inflammation to cancer (Multhoff and Radons 2012). On the other hand, intrinsic pathways driven by activation of proto-oncogenes in pre-neoplastic and neoplastic cells recruit host-derived inflammatory cells that accelerate tumor promotion and progression (Grivennikov, Greten et al. 2010). Strong links exist between inflammation and the induction of oxidative stress and genomic instability, such that it is difficult to separate out the importance of each of these mechanisms.

Characteristic 7: Immunosuppressant

Immunosuppression is a reduction in the capacity of the immune system to respond effectively to foreign antigens, including antigens on tumor cells. Persistent immunosuppression presents a risk of cancer, especially excess risk for lymphoma

when immunosuppression is accompanied by continuing exposure to foreign antigens such as organ transplants, or when it occurs in individuals who are latently infected with an oncogenic virus (Smith, Skibola et al. 2004, Hartge and Smith 2007). Immune suppression differs from other mechanisms of carcinogenesis in that agents that cause immunosuppression may not directly transform normal cells into potential tumor cells. Potentially neoplastic cells that arise naturally, or that have been transformed by other carcinogens acting by a mechanism such as genotoxicity or by the various mechanisms of action associated with oncogenic viruses, escape immune surveillance in immunosuppressed individuals. As a result, survival of these cells and their replication to form tumors is greatly facilitated by immune suppression. Several Group 1 agents included in IARC Monographs Volume 100 act entirely or largely by immunosuppression, often in concert with other Group 1 agents, especially oncogenic infectious agents. The Group 1 agents that act by immunosuppression include Human Immunodeficiency Virus (HIV-1) and the immunosuppressive drugs cyclosporin and azathioprine (Cerilli and Hattan 1974, Rafferty, Egenolf et al. 2012).

Characteristic 8: Modulate Receptor-mediated effects

Hormonally active agents that are associated with carcinogenic effects typically act as ligands via nuclear receptors and in some cases via receptors located on the cell surface. There are many other agents that may be carcinogenic by acting on receptor proteins, even though some of these also have genotoxic effects, e.g., polycyclic aromatic hydrocarbons, such as benzo[*a*]pyrene. Receptor activation broadly falls into two categories: (a) intracellular receptors that translocate into the nucleus and act on DNA as transcription factors and (b) cell surface and some intracellular receptors that activate signal-transduction pathways resulting in biological responses (Bosland 2013). However, the predominant carcinogenic effect of receptor activation is on gene transcription. Both classes of receptors can be involved in carcinogenic mechanisms, but not necessarily through activation of the receptor. Although some exogenous ligands act as agonists by competing for binding with an endogenous ligand, others may bind but lack intrinsic activating activity for the receptor they bind to and have an antagonist effect. There are also receptors for which few or no endogenous ligands have been identified, such as the aryl-hydrocarbon (Ah) receptor (Ma 2011, Baek and Kim 2014). One other important class of potential effects of exogenous agents on receptor-mediated mechanisms involves modulation of the amount of endogenous ligand available for binding and activating its receptor by affecting biosynthesis, bioavailability, bioactivation, and degradation of the bioactive ligand.

Characteristic 9: Immortalization

Volume 100 of the IARC Monographs identifies several human DNA and RNA viruses, including various human papillomaviruses, Epstein-Barr virus, Kaposi's sarcoma-associated herpesvirus, hepatitis B virus, hepatitis C virus, and human immunodeficiency virus, that are carcinogenic to humans. These viruses have evolved multiple molecular mechanisms to disrupt specific cellular pathways to facilitate aberrant replication. Although oncogenic viruses belong to different families, their strategies in human cancer development show many similarities and involve viral-encoded oncoproteins targeting the key cellular proteins that regulate cell growth (Saha, Kaul et al. 2010). Recent studies show that virus and host interactions also occur at the epigenetic level (Allday 2013). The result of these viral effects is to immortalize the target tissue cells such that they are not subject to the Hayflick limit, the point at which cells

can no longer divide due to DNA damage or shortened telomeres. For example, the Human Papillomavirus type-16 (HPV-16) *E6* and *E7* oncogenes are selectively retained and expressed in cervical carcinomas, and expression of *E6* and *E7* is sufficient to immortalize human cervical epithelial cells. E6 and E7 proteins do not possess intrinsic enzymatic activities, but instead function though a number of direct and indirect interactions with cellular proteins, a number of which are well known cellular tumor suppressors, including p53 and Rb.

Characteristic 10: Alter cell proliferation, cell death or nutrient supply

There are at least three scenarios related to cancer and cancer mechanisms in which alterations in cellular replication and/or cell-cycle control have been described. One invokes the predisposition of replicating cells for unrepaired DNA damage to lead to cancer-initiating mutations, another has attempted to identify sustained replication as a key event in various modes of action (MOAs), and a third describes the ability of a transformed cell to escape normal cell-cycle control and to continue replication. A component common to all three scenarios is the evasion of apoptosis or other terminal programming, including autophagy and pyroptosis, in at least a proportion of the cell population (Ryter, Mizumura et al. 2014). Hyperplasia alone does not necessarily lead to cancer. The inability of enhanced cell division to reliably predict carcinogenicity has been noted for some time (Melnick, Huff et al., 1993). The circumstantial context under which it occurs (e.g., sustained *vs* transient, in the presence of inflammatory mediators or genotoxicity or not) influences development of cancer after exposure to carcinogens in experimental models. Sustained cellular proliferation has been argued to be a factor in increased cancer susceptibility. As summarized in the EPA guidance assessing cancer risk from early-life exposures (U.S. EPA Cancer guidelines, 2005, "Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens"), more frequent cell division during development can result in enhanced fixation of mutations due to the reduced time available for repair of DNA lesions, while clonal expansion of mutant cells gives a larger population of mutants. For mature organisms, sustained proliferation has also been postulated to increase cancer risk using the same rationale.

Necrotic cell death releases pro-inflammatory signals into the surrounding tissue microenvironment, recruiting inflammatory immune cells to the site of trauma, which can enhance cancer-cell proliferation and promote cancer metastasis (Pollard 2008, Coussens and Pollard 2011, Coussens, Zitvogel et al. 2013). In contrast, apoptosis and autophagy have the opposite effect by removing potentially cancerous cells from a population before they acquire the changes permitting malignancy. Thus, agents which induce necrosis, apoptosis and/or autophagy can have profoundly divergent effects on cancer induction in a specific tissue.

In addition to cell death caused directly by agent toxicity, cells may die within a tumor as a result of an impaired nutrient supply. Like other proliferating cells, tumor cells require a blood supply to provide nutrients so that they can grow. Just as a neoplastic cell numbers can double exponentially, so too does its energy demand, which can quickly outstrip the supply capabilities of the existing tissue vasculature. The process of neoangiogenesis in which new blood vessels grow into a tumor is key to providing this supply of nutrients. Thus, agents that promote or inhibit angiogenesis, such as arsenic, will promote or delay tumor growth (Wang, Liu et al. 2013, Yang, Zang et al. 2014).

Cancer cells also usually show quite different cellular energetics depending on glycolysis instead of mitochondria for their supply of ATP. This is likely to be a consequence of mutation and altered gene expression rather than a cancer-inducing mechanism, but the ability of potentially carcinogenic agents to modify cellular energetics is worth noting as this may be reflective of other cancer inducing mechanisms and a switch in the cell or tissue's metabolic state.

Systematically collating and analyzing mechanistic information based on the 10 key characteristics

Step 1: Identifying the relevant information

The starting point for this systematic evaluation is to conduct comprehensive searches of the peer-reviewed literature aimed at identifying critical mechanistic studies. The searches can be constructed to address a series of study questions in the PECO (population, exposure, comparator, and outcomes) framework (Higgins and Green 2011) wherein outcomes associated with the key characteristics are identified. Specifically, the questions to be answered by the searches are, "Does exposure to the agent cause outcomes associated with one or more specific key characteristics of carcinogens"? The population (humans and any relevant experimental systems) and exposure and comparator (the agent and relevant metabolites compared to unexposed) may be sufficiently broad to enable identification of the range of available mechanistic data informative of the overall evaluation of carcinogenic hazard. This approach thus entails comprehensive, targeted literature searches using appropriate MeSH terms and key words to identify evidence on the 10 key characteristics for the agent(s) or exposure(s) under evaluation.

Additional complementary literature searches may incorporate terms for the agent and its metabolites, alone or in combination with broad terms for carcinogenicity or related effects. For instance, because IRIS toxicological reviews also encompass a range of non-cancer toxicities, "top-down" broad literature searches aimed at comprehensively identifying studies on all potential toxic effects of an agent are currently being employed. These comprehensive searches of peer-reviewed literature are supplemented by hand-searching (e.g., of past IARC Monographs or other authoritative reviews). Databases (e.g., PubChem) and peer-reviewed government reports can also be systematically searched. The search terms used and literature retrieved can be documented (e.g., using MyNCBI or https://hawcproject.org).

Step 2: Screening and organizing the results

Based on title and abstract review, studies are excluded if they no toxicological endpoints are reported (e.g., address exposure to the agent), or if no data on the chemical or a metabolite are reported. Included studies are then organized by the population (human or experimental systems) and by the outcomes associated with the 10 key characteristics (see Table 1). Studies relevant to toxicokinetics (covering absorption, distribution, metabolism and excretion) are also identified. Additionally, authoritative, balanced review articles are identified, as are studies reporting toxicological endpoints in cancer target (e.g., liver toxicity for a hepatocarcinogen) and non-target (e.g., neurotoxicity) tissues. These may include morphological evaluations pertaining to the dysfunction of organs, tissues, and cells. Studies may fall under multiple categories.

Step 3: Summarizing the evidence

The final step is to summarize the extent of data available, addressing the range of study designs and doses tested, whether effects are observed at the physiological, cellular or molecular level, and any consistencies or differences in results within and across experimental paradigms. Emphasis is given to human data, where they exist, consistent with guidance (e.g., in the IARC Preamble and the US EPA Cancer Guidelines). Gaps in evidence are identified.

Identifying evidence on multiple carcinogenic mechanisms: benzene case example

The number of mechanisms by which chemicals are known to contribute to carcinogenesis can be extensive if one includes all biochemical or molecular endpoints, but they can be grouped into a limited number of categories (e.g., genotoxicity, immunosuppression, etc.). Guyton et al. described 15 types of "key events" associated with carcinogenesis that collectively represented the majority of known carcinogenic modes of action (Guyton, Kyle et al. 2009). The experts present at the first of the IARC meetings in 2012 originally identified 24-25 mechanistic endpoints with several subcategories in each. This was considered too many and it was determined that a number of them could be merged. When this was done at the second meeting in 2012 it was concluded that carcinogens commonly show one or more of 10 key characteristics which are listed in Table 1. These represent the majority of established carcinogenic mechanisms.

It is increasingly evident that multiple biological alterations or sets of different perturbations are necessary to convert a normal cell to a transformed cell and ultimately a tumor (Hanahan and Weinberg 2011). Carcinogens appear to impact this complex process in multiple ways and can act through multiple mechanisms of action to induce cancer and other adverse health outcomes. To illustrate this point, we have evaluated the evidence for which key characteristics contribute to the carcinogenicity of benzene, a Group 1 carcinogen, in humans and animals.

Mechanistic studies were identified from targeted literature searches to identify outcomes of benzene pertinent to the key characteristics, in populations comprising humans or experimental systems. Specifically, literature searches were constructed to address the following outcomes: 1) genotoxic endpoints (comprising the first three key characteristics); 2) epigenetic effects; 3) oxidative stress; 4) inflammation or immune effects (comprising key characteristics 6 and 7); 5) receptor-mediated effects; and 6) alterations in cell proliferation or death or nutrient supply. The literature searches were conducted using the HAWC Literature Search tool (https://hawcproject.org/), documenting the search terms, sources, and articles retrieved. Following title and abstract review, studies were excluded if they were not about benzene or its metabolites, or if they reported no data on toxicological endpoints (e.g., concerned exposure measurements). Included studies were further sorted into categories based on the mechanistic endpoints evaluated. Reviews or commentaries were identified, as were studies reporting toxicological endpoints in cancer target and non-target tissues. Studies reporting multiple endpoints, or evaluating human and non-human samples, commonly received more than one mechanistic tag. A separate category was used for studies (e.g., using microarrays) that simultaneously evaluated a range of multiple

characteristics. The resulting graphical displays (e.g., literature trees) documents the number of studies identified, excluded and categorized per mechanistic topic and species (see Figure 1).

The results are shown in Table 2, where the evidence for a particular characteristic is classified on a 2, 1, 0 scale where 2 = Sufficient evidence; 1 = Limited evidence; and, 0 = Insufficient evidence. For benzene, the evidence was judged sufficient that metabolic activation, genotoxicity and immunosuppression are established mechanisms of carcinogenicity in both animals and humans (McHale, Zhang et al. 2012) (Table 2). There is insufficient or no evidence that inflammation and immortalization play a role in benzene carcinogenicity (Table 2). However, limited evidence exists for 5 of the other key characteristics in humans. This suggests that there is sufficient or limited evidence for benzene causing 8 of the key characteristics of human carcinogens and that they are consistently observed, for the most part, in both humans and experimental animals (Table 2). These 8 characteristics can be integrated into a single overarching mechanism or adverse outcome pathway network as described by McHale et al (2012) and in the EPA's NexGen Risk Assessment Report.

Conclusions

Identification and incorporation of important, novel scientific findings providing insights into cancer mechanisms is an increasingly important aspect of carcinogen hazard identification. Systematic approaches to the search, evaluation and inclusion of peer-reviewed literature are needed to facilitate comprehensive inclusion of evidence covering the range of available mechanistic data informative of the overall evaluation of carcinogenic hazard. Ten key characteristics of human carcinogens were identified during the Volume 100 Monographs and two subsequent expert workshops. These characteristics, while not representing mechanisms or modes of action themselves, provide the rationale for an objective approach to identifying and organizing relevant mechanistic studies. Accordingly, the mechanistic discussion in IARC monographs has been restructured to more explicitly analyze evidence for these key characteristics of carcinogens. A case study on benzene was conducted to illustrate the utility of the approach. This example revealed that the approach identified pertinent literature on mechanisms, and provided a practical, objective method for organizing the identified evidence. As this approach is carried forward, it will facilitate the objective identification of mechanistic data for consideration in the context of epidemiology and animal bioassay evidence of carcinogenicity in classifying agents with regard to carcinogenic hazard. These developments will aid advancement of future evaluations of newly introduced chemicals, including those for which mechanistic data provide the primary evidence of carcinogenicity.

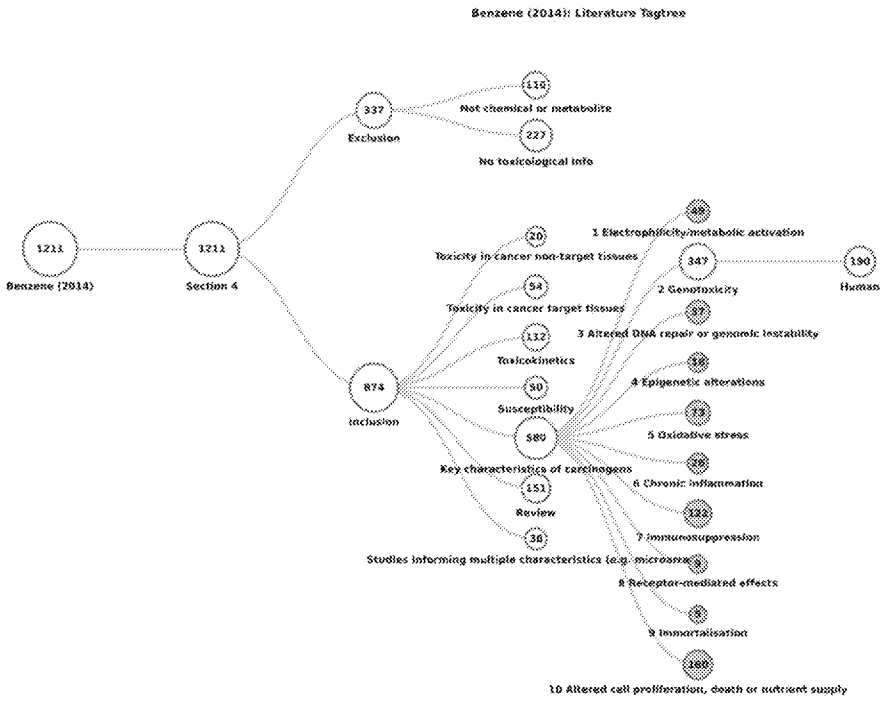
Table 1: Key Characteristics of Carcinogens

Characteristic[1]	Example relevant evidence	Commonly Linked Characteristics[2]
1. Electrophilic or ability to undergo metabolic activation	Parent compound or metabolite with an electrophilic structure (e.g., epoxide, quinone, etc), formation of DNA and protein adducts.	2,3,4,7,8,9
2. Genotoxic	DNA damage (DNA strand breaks, DNA-protein cross-links, unscheduled DNA synthesis), intercalation, gene mutations, cytogenetic changes (e.g., chromosome aberrations, micronuclei).	1,3,4,5,10
3. Alter DNA repair or cause genomic instability	Alterations of DNA replication or repair (e.g., topoisomerase II, base-excision or double-strand break repair)	1,2,4,6,7,9,10
4. Epigenetic alterations	DNA methylation, histone modification, microRNAs	1,6,10
5. Oxidative stressor	Oxygen radicals, oxidative stress, oxidative damage to macromolecules (e.g., DNA, lipids)	2,6,8,10
6. Induce chronic inflammation	Elevated white blood cells, myeloperoxidase activity, altered cytokine and/or chemokine production	3,4,5,7,8,10
7. Immunosuppressant	Decreased immunosurveillance, immune system dysfunction	1,3,6,8,9
8. Modulate receptor-mediated effects	Receptor in/activation (e.g., ER, PPAR, AhR) or modulation of exogenous ligands (including hormones)	1,5,6,7,10
9. Immortalization	Inhibition of senescence, cell transformation	1,3,7,10
10. Alter cell proliferation, cell death and nutrient supply	Increased proliferation, decreased apoptosis, changes in growth factors, energetics and signaling pathways related to cellular replication or cell cycle control, angiogenesis	2,3,4,5,6,8,9

[1] Colors in this column indicate characteristics for which an individual working group or committee member or group of members work together to identify data and draft the initial language. Some mechanisms can cause cancer without other mechanisms, but generally it is the combined effect of multiple mechanisms that will be the root cause.

[2]Any of the 10 characteristics in this table could interact with any other, but there are some common interactions that are highlighted in the column (e.g. oxidative stress, DNA damage and chronic inflammation when combined provides stronger evidence for a cancer mechanism than would oxidative stress alone)

Figure 1: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4 Epigenetic alterations
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7 Immunosuppression
8
8 Receptor-mediated effects
6
9 Immortalisation
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10 Altered cell proliferation, death or nutrient supply

Table 2: Key characteristics of carcinogens – Benzene as an example with a 2,1,0 scale

2 = Sufficient evidence
1 = Limited evidence
0 = Insufficient evidence

Characteristic	IARC 100F	Human	Animal
Metabolic activn./electrophilicity	2	2	2
Genotoxicity	2*	2	2
Altered DNA repair / genomic instability	1	1	1
Inflammation	0	0	0
Oxidative stress	1	1	1
Receptor-mediated effects	1	1	1
Altered cell proliferation or death	1	1	1
Immunosuppression	2	2	2
Epigenetic alterations	1	1	0
Immortalization	0	0	0

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